1. Gaafar2006 (truncated)

STIMULATION AND CONTROL OF *E. COLI* BY USING AN EXTREMELY LOW FREQUENCY MAGNETIC FIELD

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Abstract. The effect of a 50 Hz magnetic field of strength 2 mT on each of the growth characteristics, the antibiotic sensitivity and the ultra structure of *E. coli* bacteria cells have been studied. Equal volumes of *E. coli* cells were exposed to the magnetic field for different periods, the two most effective periods, namely, 6 h and 16 h were chosen for all our experimental studies. The results indicated that exposure of the microorganisms to the demonstrated magnetic field caused pronounced changes in the growth characteristic curves, where a suppressive effect was observed on the cell growth and the number of cells at stationary phase markedly decreased after exposure period of 6 h but there was a slight increase in the growth rate after exposure period of 16 h with increase in the number of cells. Further, changes in the antibiotic sensitivity was observed after exposure period of 6 h since *E. coli* cells became more sensitive to certain antibiotics such as amoxicillin, nalidixic acid and erythromycin as revealed in the increase in their zone diameters while, after a 16 h exposure period, it became more resistant to the same antibiotics. Furthermore, the results of the ultra structure showed that while exposure period 6 h decreased the cell length, the exposure period 16 h elongated the cell length with decreasing the thickness of the cell wall beside the disappearance of the majority of cytoplasmic components.

Keywords: electromagnetic field, *E. coli,* growth rate, antibiotic sensitivity, ultra structure.

INTRODUCTION

During the past few decades, due to the increasing consumption of electric energy in industry, medicine, research, communication systems and household electric appliances, the level of exposure of biological systems to electromagnetic fields has grown by orders of magnitude over a wide frequency range extending from 0 to 100 GHz. For example, hair dryers, electric shavers and electric hand tools may expose the user to magnetic fields of several times above the background.

Received July 2006;
in final form September 2006.

ROMANIAN J. BIOPHYS., Vol. 16, No. 4, P. 283–296, BUCHAREST, 2006

The extremely low-frequency (ELF) electromagnetic field (EMF) exists in all occupational and residential environments. Some scientists allege that exposure to magnetic fields generated by power delivery systems is responsible for certain diseases; therefore, it is both appropriate and important to evaluate the possible effects of man-made electromagnetic field on living organisms. Fadel *et al.* [7] reported that the main damaging role of the 50 Hz magnetic fields might be on the cellular membrane that strongly affects, not only the cellular physiological functions, but also the cell-to-cell communications.

Ma Haile *et al*. [15] studied the effect of pulsed magnetic field intensity and pulse number (PMF) on bactericidal property of PMF in sterilization of fresh watermelon juice. Their results showed that the overall bactericidal effect was strengthened as the magnetic field intensity and pulse number increased with the best effect observed when the magnetic field intensity was 2.53 T and pulse number was 20.

Piatti *et al*. [23] found that the exposure of *Serratia marcescens* to a static magnetic field of 80 ± 20 Gauss resulted in the inhibition of *S. marcescens* growth. Dacosta [5] and Barnickel [3] used new non-destructive decontamination technique to reduce the bacteria in milk, orange juice and also in cheese. Pulsed electric field, pulsed magnetic field and pulsed light were used.

Fojt *et al*. [8] found that *E. coli, Leclercia adecarboxylata* and *Staphyloccus aureus* viability was affected with the magnetic field (10 mT, f = 50 Hz) they also found that the decrease of the colony forming units (CFU) starts immediately after the magnetic field was switched on. Mei *et al*. [16] studied the inactivation of microorganisms by a pulsed magnetic field. It was reported that the application of electromagnetic pulses evidently causes a lethal effect on *E. coli* cells suspended in buffer solution.

Shengying *et al*. [31] studied the non-thermal sterilization by using the self-designed generator of magnetic field. The results showed that the magnetic flux density, which had the greatest effect on *E. coli*, was 1 T. The greatest destruction rate of *E. coli* was 78% under 8 hours of magnetic field (1 T) treatment.

Also, Mohamed *et al*. [18] reported that exposure of the microorganism *S. typhi* to the magnetic field (10, 20 G for a period of 2 hours) caused pronounced changes in the growth characteristics and the number of cells at the stationary phase increased.

Electromagnetic fields are also used in therapy to enhance the transdermal drug delivery [21]; in certain dairy industry to manipulate growth characteristics of yogurt culture, where the change in culture metabolism rather than an elevation in the overall bacterial population, was induced by a 60 Hz, 4.3 G EMF [17]; in soil studies and inactivation of indicator bacteria of cattle slurry by exposure of 400 – 700 g for 60 seconds to magnetic field (380 V, 50 Hz) [10]; in some food

preservation to control certain pathogenic bacteria such as *Salmonella* and *E. coli* contaminated meat samples [4]; in agriculture to improve soil fertility by increasing the nodulation process by exposure of Rhizobium sp. to a low strength (5×10^{-3} T) EMF before inoculation to mycorrhizal chick-pea seedlings [2].

Finally, EMF has been used either to inhibit or to stimulate the growth rate of microorganisms under appropriate conditions [10].

This work is concerned with the study of the biological effect of magnetic fields, as a component of the non-ionizing radiations, on a unicellular system. Pathogenic microorganisms, especially *Escherichia coli,* are chosen to be our experimental model for many reasons; it is widely distributed in the environment such as soil, water and air. *E. coli* is a member of the normal intestinal flora of humans. It causes several diseases such as urinary tract infection, wound infection, traveller's diarrhea. It reaches blood stream and causes sepsis and meningitis [25]. *E. coli* are rapidly growing, Gram-negative, rod-shaped cells measuring approximately 0.5×2 µm length [20].

In the light of the pathogenic effects of these bacteria, we aim, here, to study the effect of different exposure periods to a 20 G, 50 Hz magnetic field on the cell activity with the aim to control the activity through the exposure period. Moreover, we intend to take two exposure periods for investigating the effect of such a magnetic field on the growth rate, the antibiotic sensitivity and the ultrastructure of the exposed cells.

MATERIAL AND METHOD

BACTERIAL STRAIN

Escherichia coli ATCC ≠ 25992 was cultivated over night on Nutrient broth at 37 °C; each ml of bacterial suspension contained 13×10^3 CFU/ml.

SURVIVAL CURVE

To study the bacterial growth, a standard survival curve was plotted between the absorbance of volume A (unexposed cells) at 600 nm and the concentration of cells (number of cells / mL). For cell counting the plate count technique was used [28]. Appropriate dilutions of the bacterial cells were used to inoculate nutrient agar plates. Inoculated plates then incubated at 35 °C for 24 h by counting the number of colonies developed after incubation and multiplying it with the dilution factor the number of cells in the initial population is determined.

MAGNETIC FIELD EXPOSURE FACILITY

Bacteria volumes were exposed to a homogeneous magnetic field generated by a solenoid consisting of 320 turns from electrically insulated 2 mm copper wire

wound in a homogeneous way around a copper cylinder 1.5 mm thick, 40 cm diameter and 25 cm length. The cylinder wall was earthed to eliminate the electric field components effects. The magnetic field generator was temperature controlled during the exposure period by using a water pump as shown in Fig 1. The temperature during the exposure period was 37 °C. The tubes of the exposed bacteria were put in the middle of the coil by using supports inside it to get a homogeneous and higher magnetic fields strength. The ends of coil are connected to variac fed from the mains (220 V, 50 Hz). The field strength was 20 G and adjusted by changing the voltage through the coil.

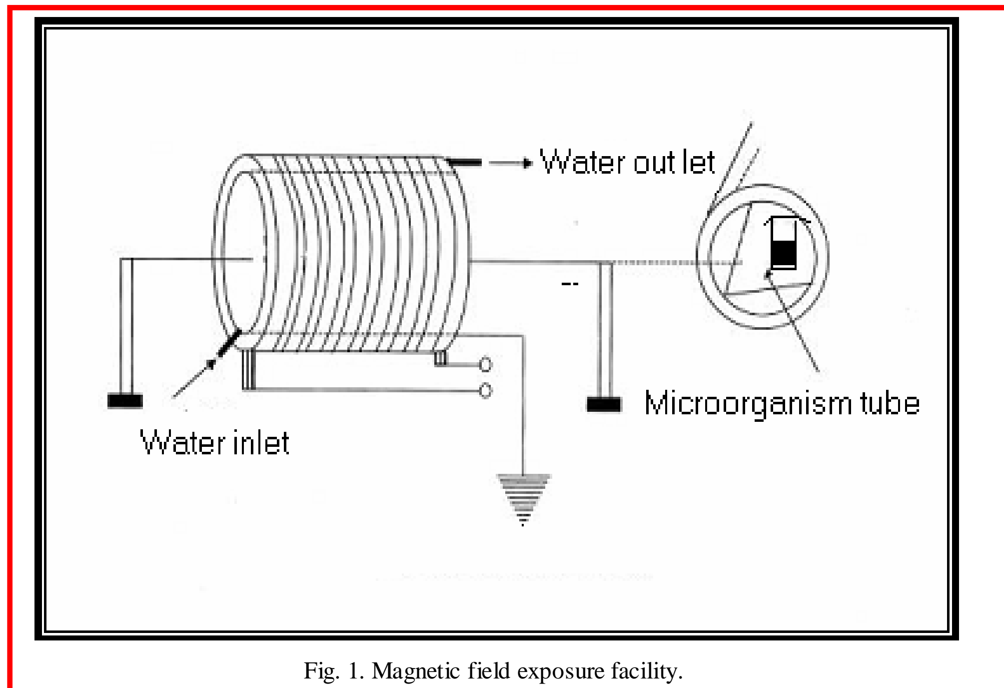

Fig. 1. Magnetic field exposure facility.

GROWTH CHARACTERISTICS

Ten volumes from the strain were incubated for 18 h and then exposed to different exposure periods. The first volume was exposed for two hours; the second volume was exposed for four hours; the third volume was exposed for six hours and so on until 20 h. For each exposure volume, there was a corresponding control volume. Through measuring the absorbance of every volume, the two volumes exposed to the two periods, 6 h and 16 h, were chosen for additional investigations concerning the growth rate, the antibiotic sensitivity and the ultra structure of the cells due to their high effects.

Four volumes were used in this study: A, B, C, and D. Volume A is the control of volume B exposed to 6 h magnetic field, volume C is the control of volume D exposed to a 16 h magnetic field.

GROWTH RATE

The growth rates of all volumes (A, B, C and D) were determined through measuring the absorbance of the viable cells after 2, 4, 6 until 24 h. The absorbance of the volumes was measured and then plotted as a function of time. Spectronic 20+ Series Spectrophotometer (USA) was used for this purpose.

ANTIBIOTIC SUSCEPTIBILITY TEST *IN VITRO*

The isolated *E. coli* cells were tested for their *in vitro* susceptibility to various antibiotics such as erythromycin 10 µg, chloramphenicol 30 µg, cefodoxil 30 µg, nalidixic acid 30 µg, garamycin 10 µg, and amoxicillin 25 µg by disk diffusion test according to Baker *et al*. [1].

The antibiotics used in this study were chosen to be with different modes of action. The diameters of the inhibition or stimulation zone of the volumes A, B, C, and D were measured after 24 h from the exposure process.

ULTRA STRUCTURE OF BACTERIA CELLS

Volumes A, B, C and D were prepared for the transmission electron microscope by the method recommended by Philipe [22] to define the changes in the morphological structure of *E. coli* cells.

RESULTS AND DISCUSSION

The results obtained in this work concern the induced changes in the structure and the characteristic behavior of *E. coli* resulting from the exposure to the demonstrated magnetic field. These results may be of a great importance for evaluating the benefits as well as the hazards of the exposure to the low frequency low-level magnetic field.

Also the importance of this work lays in the fact that *E. coli* as a microorganism is a unit cell behaving as a complete alive biological system.

SURVIVAL CURVE

Fig. 2 shows the variation of the number of microorganisms in CFU/ml as a function of the sample absorbance measured at 600 nm. The results show the linear dependence of the absorbance on the number of microorganisms in CFU/ml. By using this relation we can calculate the number of the microorganisms/ml (*C*) from

the measured value of its absorbance (A). The relation can easily express the linear dependence:

$$C = 9.7 \times 10^{9} A \quad (1)$$

This curve has two supplimentary points vs that from Figure 2 of ***Article***

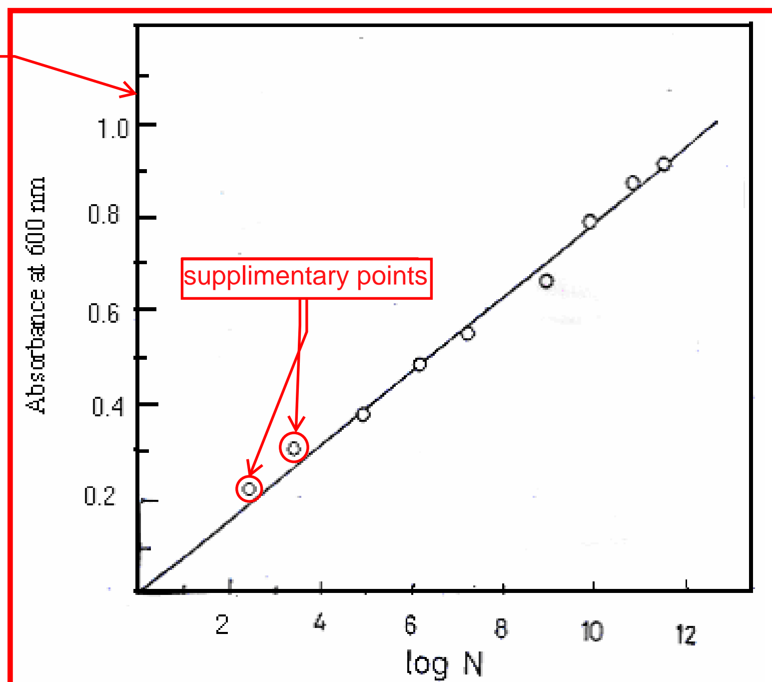

Fig. 2. Survival curve between log number of bacteria cells/ml and the absorbance at 600 nm.

GROWTH CHARACTERISTICS CURVE

Fig. 3 shows the change in the absorbance of bacterial strain as a function of the time of exposure to the magnetic field.

It is clear from this figure that the exposure periods 2, 4, 6, 8, 10 and 12 hrs decreased the absorbance and, in accordance with equation (1), indicate a decrease in the cells number and consequently an inhibition case for the bacteria. However, at the exposure periods of 14, 16, 18 and 20 h the increased absorbance relative to their control indicates an increase in the cells number and a stimulation case. These results are in a good agreement with Mohamed *et al.* [18] where the number of cells of *S. typhi* microorganism exposed to 20 G magnetic fields for 2 hours increased relative to those unexposed.

Also, Jaffe [10] reported that the electromagnetic field was used either to inhibit or to stimulate the growth of the microorganism under appropriate conditions.

For this reason we used the exposure period of 6 h (volume B) as an inhibition case where the number of cells was 10^8 and became 10^7 cells/ml also the exposure period of 16 hours (volume D) as stimulation case where the number of cells was 3.5×10^2 and became 3.5×10^4 cells/ml. Moreover, we intend to take the two exposure periods for investigating the effect of the magnetic field (20 G, 50 Hz) on the growth rate, the antibiotic sensitivity and the ultrastructure of the exposed cells.

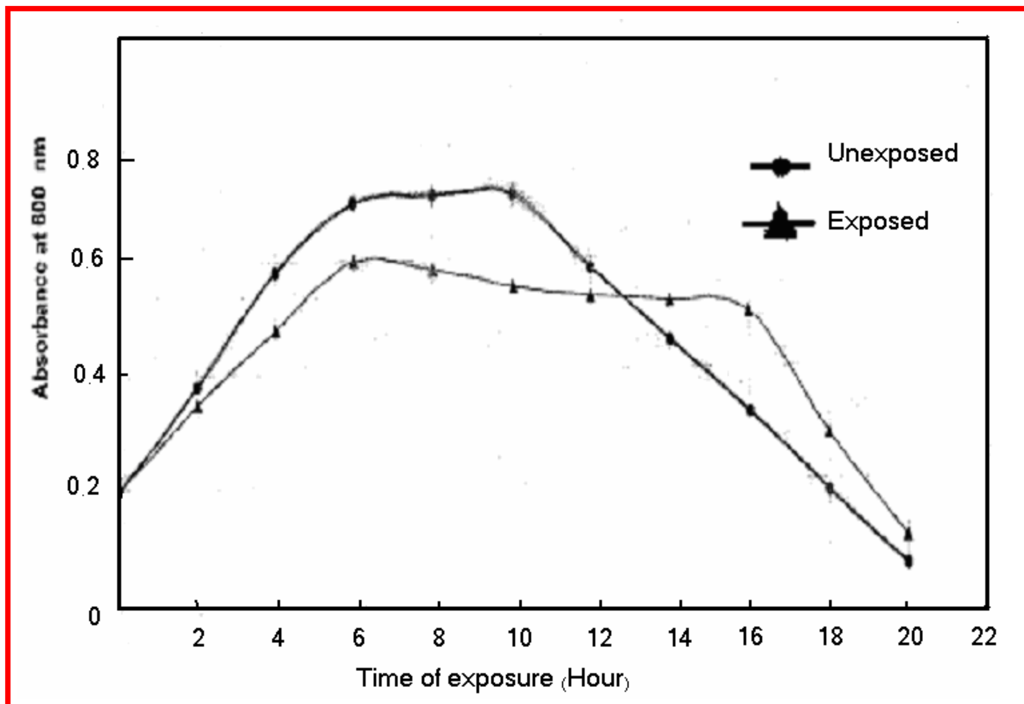

Fig. 3. Absorbance at 600 nm of *E. coli* cells at a different exposure periods.

EFFECT OF MAGNETIC FIELD ON BACTERIAL GROWTH RATE

Fig. 4 shows the growth rate of volumes A and B. It is clear from the figure that there is a decrease in the growth rate of the *E. coli* cells exposed to 6 h relative to its unexposed ones.

Fig. 5 explains the growth rate of volumes C and D. It is clear from the figure that there was a slight increase in the growth rate of the exposed *E. coli* cells relative to its unexposed.

The results in Figs. 4–5 and the calculated data from these curves in Table 1 indicate considerable changes in the growth curve characteristics for the two exposure periods 6 and 16 hrs. For the exposure period of 6 h (volume B) the maximum growth occurred at 16 h while for the unexposed cells at 18 h; also, the

maximum number of microorganisms decreased to be 2×10^{7} cells/ml as compared with the unexposed cells 8×10^{9} cell/ml. These results are in a good agreement with M. Li *et al.* [12] who used magnetic field for 4 h (0.2 kWh/m^{2}) to decrease the survivability of *E. coli* to reach 0.01%.

However, for the exposure period of 16 h (volume D) the maximum growth occurred at 14 h with increasing the maximum number of the microorganism to be 2×10^{10} cells/ml as shown in the table. Moreover, from these results one sees how the period of the active growth (log phase) decreased for the two volumes B and D, which became 12 and 10 h, respectively, while it was for the unexposed cell 14 h and also the lag phase was short. In spite of these facts, the exposure period of 16 h increased the cell division rate in a good agreement with Nascimento *et al* [19] who concluded that the electromagnetic field (8 h, 5 G, 60 Hz) had a positive effect in the consume of glucose and growth of *E. coli.* They attributed the increase in the growth to the shortening of lag phase and excitement of log phase.

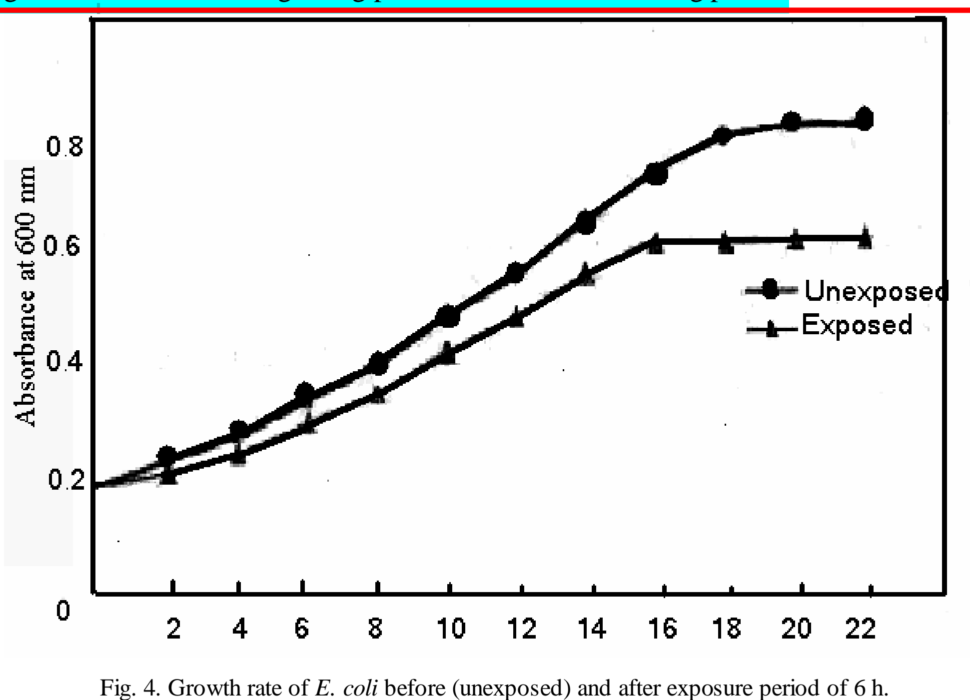

Fig. 4. Growth rate of *E. coli* before (unexposed) and after exposure period of 6 h.

Potenza [24] suggested that exposing *E. coli* cultures to 300 mT static magnetic field may stimulate transposition activity.

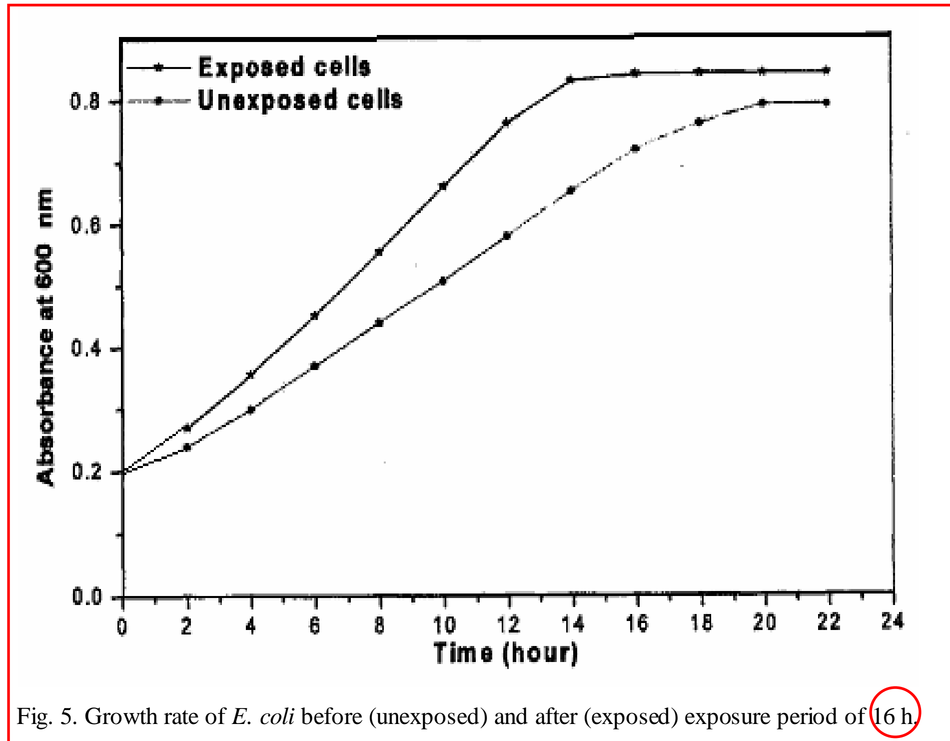

Fig. 5. Growth rate of *E. coli* before (unexposed) and after (exposed) exposure period of 16 h.

Table 1

Growth characterization of *E. coli* before and after exposing to the magnetic field

Samples	Log phase (h)	Stationary phase (h)	No. of cells/ml at stationary phase
Volume A	14	18	8×10^9
Volume B	12	16	2×10^7
Volume C	14	18	7×10^9
Volume D	10	14	2×10^{10}

The inhibitory effect of EMF after an exposure period of 6 h on the growth of bacteria may be due to the interaction between electric charges induced by EMF and that of the cytoplasmic membrane resulting in partial abolishment of electric potential of the cytoplasmic membrane with a subsequent decrease in the macromolecular biosynthesis. Also EMF may cause damage of bacterial DNA and inhibition of its replication [9, 14, 27].

Since the present data proved the cellular membrane of the microorganism had been affected by the external magnetic field, then one expects a disturbance in their metabolic activity and, consequently, a change in their cell division in a good agreement with Mohammed *et al.* [18] who reported that exposing *S. tyhi* to a 20 G magnetic field increased their cell division and cell number.

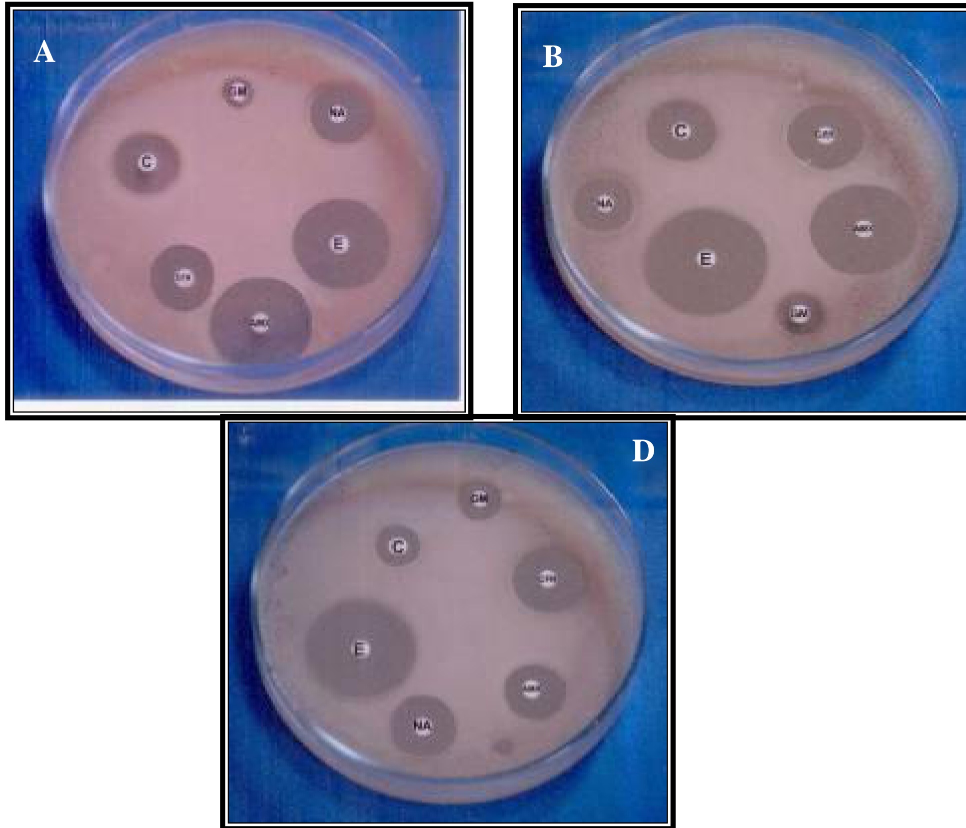

Fig. 6. The antibiotic zones for the unexposed and exposed bacteria (6 h and 16 h, respectively).

EFFECT OF MAGNETIC FIELD ON ULTRA STRUCTURE OF BACTERIA CELLS

Fig. 7 shows the ultra structure of *E. coli* cells for the three volumes, A, B and D. The figure illustrates a complete lyses of the cell wall without destruction of cytoplasmic membrane, granular ribosomal distribution and no vacuoles appear in the cytoplasm for volume B. But for volume D, an elongation of the cells was observed with an increase in the wall thickness of cell and the majority of the cytoplasmic component disappeared.

Strasak *et al.* [30] found that magnetic field effects depend on the cells shape.

To get better understanding of the interaction mechanism of the magnetic field with biological systems an understanding of the bioelectrical signals resulting from the biological system during metabolic activity is required.

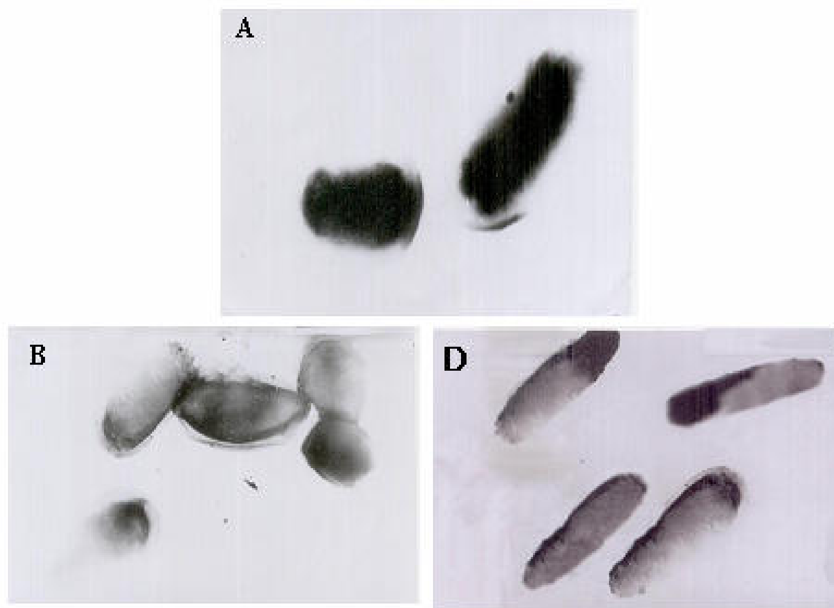

Fig. 7. Ultrastructure of the unexposed and exposed bacteria cells (magnification 20×10^{3}).

Mohamed *et al.* [18] reported that the bioelectrical signals from the microorganism normally were carried out through bending of their cellular membranes, which generate an electric impulse through a phenomenon known as flexoelectricity. The amplitude and the frequency of these impulses depend on the amount and frequency of bending. These impulses travel through the medium separating the microorganisms and were received by the signal receptors at the surface and that impeded in the cell membrane. Therefore the flexibility of the membrane is the most important parameters for generation of these signals. Also mentioned is that the biomagnetic field from the biological system associating to the bioelectrical signals from the membrane of the cells through its metabolic function is very weak, in nanogauss range (20×10^{-8} G). When the biological systems exposed to an external magnetic field whose strength is very large relative to the biomagnetic field of the cells a disturbance in their metabolic function will be expected and lead to death of the cells or to the increase of their cell division [7, 26]. Del-Re *et al*. [6] found that *E. coli* bacteria that had been exposed for a long time to a 50 Hz, low intensity (0.1–1 mT) magnetic field gave colonies with significantly lower transposition activity compared to sham-exposed bacteria. Such reduction in transposition activity was positively correlated to the intensity of the

EMF, in a dose effect manner also Zhang *et al.* [32] concluded that strong SMF induce mutations through elevated production of intracellular super oxide radicals in *E. coli* cells.

From the present data it is easily deduced that the cellular membrane of the microorganism had been affected by the external magnetic field in a good agreement with Fadel *et al*. [7]. Then we can expect the disturbance of cell division and hence, a change in the number of the cells per ml or the measured change in the membrane sensitivity to antibiotic demonstrated also the change in the internal structure of the cells.

CONCLUSION

From this work, it is concluded that the electromagnetic field (20 G) affected considerably the virulence of *E. coil* cells. 6 h exposure time was found to cause an inhibition case whereas 16 h exposure time enhanced the virulence.

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2. Hanafy2006 (truncated)

ELECTRIC FIELD AFFECTED THE MOLECULAR STRUCTURE OF THE TOTAL SERUM PROTEINS OF THE MICE BLOOD

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Abstract. An electric field of 6 kV/m strength and 50 Hz frequency was directed horizontally to three groups of mice for exposure periods 30, 45 and 60 days respectively and for 30 days post exposure. The dielectric properties of the total serum proteins of the exposed mice were studied as an indication of the effect of the electric field on the molecular structure of the serum proteins. The molecular structure of the total serum proteins were studied through measuring their dielectric relaxation and the electric conductivity in the frequency range 0.1 – 5 MHz at 4 ± 0.5 ºC. The absorption spectra of the extracted proteins were also measured in the wavelength range 200 – 600 nm. The results showed that the electric field lowered the permittivity value of the serum proteins and increased its conductivity; a fact that indicates pronounced changes in the molecular structure of the total serum protein of the exposed mice. In addition, the intensity of the absorption spectral bands of the serum proteins of the exposed mice was found to decrease.

Key words: low frequency electric field, total serum proteins, dielectric relaxation.

INTRODUCTION

Possible health effects of exposure to low frequency low intensity electric and magnetic fields are receiving increased interest in the scientific literature. The increasing scientific interest with the effect of electric field on leaving cells during recent decades is mainly attributed to its guide in throwing light on major unsolved biological problems such as irregular cell division Winterhaller [23]

Eman *et al.* [1] showed changes in the dielectric relaxation and electric conductivity of the extracted protein molecules of the exposed mammalian eye (5 kV/m).

Ibrahim [8] stated that application of small d.c. electric field intensities (1–5 V/cm) on erythrocytes increased their electric conductivity.

On the other hand, Walter *et al.* [21], Macginitic *et al.* [12], and Laberge [9] proved that electric field inhibited the biological properties of the cells membrane protein. The Biological effects of such a field (6 kV, 50 Hz) on the bone marrow of

Received October 2004;
in final form July 2006.

ELECTRIC FIELD FACILITY

Extremely low frequency of electric field strength of 6 kV/m and frequency of 50 Hz was generated between two parallel aluminum electrodes of dimensions 60×50×0.2 cm fixed vertically at the two vertical sides of the mice cage (Fig. 1).

The electric field was derived directly from 50 Hz high voltage step-up transformer, manufactured by the Center of Scientific and Electronic Equipment Maintenance, Faculty of Science, Cairo University. The design of the apparatus is shown in Fig. 1.

EXPOSURE PROCESS

Twenty five male mice were exposed as follows: group 1 was used as control, groups 2, 3 and 4 of mice exposed to periods 30, 45 and 60 days respectively. Group 5 was investigated after a period late 30 days from switching off the power supply.

PREPARATION OF TOTAL SERUM PROTEINS

From each mouse, one ml of peripheral blood was obtained by micro-haemotocrite tube from ocular vein of living mice and centrifuged at 3000 rpm for 30 min. After centrifugation the supernatant serum was removed carefully with a micropipette. The total protein in serum was estimated by means of Biuret reaction, according to the technique of Weichselbaum [22] and transferred to new eppendorf tubes and kept in deep freezer until use.

THE DIELECTRIC MEASUREMENTS

The extracted total serum proteins were diluted with bidistilled water at a ratio 1:20 by volume. Loffler *et al.* [10] calculated the dielectric properties of a protein and its solvent and they found that the coupling between the dielectric relaxation of the peptide and that of the water component is particularly important for correctly describing the dielectric constant of the peptide.

The dielectric relaxation of the extracted proteins were measured in the frequency range 100 kHz to 5 MHz using a Loss Factor Meter type 1033, R.F.T., Funkwerk, Erfurt, Germany, and a cell type PW 950/60 manufactured by Philips Holland. The cell has two parallel squared platinum black electrodes of 0.8 cm side each, 64 cm^2 area (A), and 1.0 cm separation distance (d).

Dielectric measurements for the samples were carried out at fixed temperature of 4 ± 0.5 °C using an incubator type 2771 Kattermann, Germany. The relative permittivity (dielectric constant), ε', of the sample is defined as the ratio of

the capacity measured with the sample to that measured by the cell in vacuum. The dielectric loss ε" is the part of the energy of an electric filed that dissipated irrecoverably as heat in the dielectric. The values of relative permittivity ε' for the samples were calculated at each frequency from the measured value of their capacitance through the relation

$$\varepsilon' = \frac{Cd}{\varepsilon_0 A} \tag{1}$$

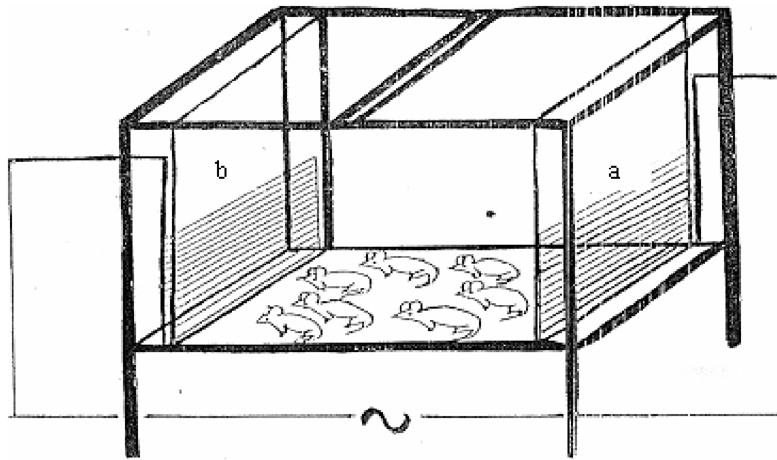

Fig. 1. Electric field facility (a, b aluminum plates).

The dielectric loss, ε", and the conductivity were calculated from measurements of the sample capacitance and resistance [6].

The difference between the values ε'_s and ε'_∞ at low and high frequency is called the dielectric increment, Δε', i.e. this quantity is a measure for the shape and volume of the nonpolar solution consisting of proteins and bound water [7]. The spectrum of biological macromolecules such as protein at high frequency (hundreds of kHz region) is known as β dispersion, which comes from the polarization of protein and other organic macromolecules [3].

Moreover, the a.c. conductivity S (s^{-1}) was calculated from equation (2) [18].

$$\mathrm{S} = \frac{\sigma}{\varepsilon_0} = \omega\varepsilon'' = 2\pi f \varepsilon'' \tag{2}$$

where ε_0 is the permittivity of free space and σ is the real conductivity.

The average molecular radius of the protein molecule was estimated from the relation [18]:

$$r^3 = \frac{kT\tau}{4\pi\eta} \tag{3}$$

where k is the Boltzmann constant, T is the absolute temperature, η is the viscosity of the protein solution and τ the relaxation time, namely, the time at which the dielectric molecule has the ability to relax under the effect of the applied field and calculated from the relation:

$$\tau = \frac{1}{2\pi f_c} \tag{4}$$

f_c being the critical frequency corresponding to the mid-point of the dispersion curve (or the frequency at the maximum loss). The accuracy of the experimental set-up was about 1 – 3% in the whole frequency range investigated.

RESULTS

Fig. 2 illustrates the variation of the permittivity (dielectric constant) ε' as a function of the frequency for the total serum protein of five mice groups. It is clear from the figure that the permittivity ε' passed through a dielectric dispersion [4] and the decrease in the values of ε' was accompanied by an increase in the value of conductivity S, which we considered as indicating confidence in the measurements. It is clear also that the dielectric increment $\Delta\varepsilon'(=\varepsilon'_s - \varepsilon'_\infty)$ for the exposure periods 30 day is lower than the other periods and increased by increasing it.

The changes in the value of $\Delta\varepsilon'$ were attributed to change in shape and volume of the nonpolar solution consisting of protein molecules [7].

Fig. 3 shows the variation of the dielectric loss ε'' as a function of the frequency for the all the total serum proteins samples.

It is clear from the figure that the middle point of the dispersion curve (at the critical frequency f_c) was changed from one treatment to another as compared with the control sample and then resulted in changes in the relaxation time τ of the samples (Eq. 4). Fig. 4 shows the variation of the conductivity S (s^{-1}) $=\left(\frac{\sigma}{\varepsilon_0}\right)$ for all the groups as a function of the applied frequency. It is clear that the electric conductivity of the total serum proteins molecules of the exposed groups is larger than the control group.

fact that the dielectric dispersion at low frequency does not follow the typical Deby relaxation curve [17]. It was reported that this magnitude of έ is due to counter-ion polarization.

The presence of a dielectric dispersion in the frequency range 10^5 – 10^7 for the protein used agrees with the previous finding of Grant [4] for other types of proteins.

Also, the slight increase in the relaxation time and consequently in the average molecular radii of the extracted protein molecules from the exposed groups relative to the unexposed explained that the shape and the volume of the protein molecules are changed [7].

In addition to that, there is a decrease in the values of $\Delta\varepsilon'$ for the blood serum proteins of the exposed mice. Since these changes in $\Delta\varepsilon'$ are functions of changes in the dipole moment of the macromolecules which will consequently depend on the center of mass of the charge distribution and the molecules radius [7], one may conclude that there are some biophysical processes running within the protein molecules resulting from the interactions of the electric field which may cause rearrangement of its charge distribution and hence changing its properties. This result is in a good agreement with. Mccammon [13] who reported that the variation in the surface charge may cause the enzyme receptors to be more sensitive to potential changes.

Also, Pitera *et al.* [16] showed that the behavior of charged residues is the primary determinate of the effective permittivity.

On the other hand, the electric conductivity of the exposed groups became larger than the unexposed. This result has been interpreted before by Sanders *et al.* [19] who suggested that exposure to electric field induces changes in the electrical potential across cell membrane and produce electrically excitable cells.

Also, Ibrahim [8] showed that the increase in the electric conductivity of the exposed cells is due to the changes in the dipoles orientation of their membrane components, which lead to conformational changes in the membrane structure.

The genotoxic effects of extremely low frequency or power (50 – 60 Hz) have been investigated in a variety of systems, most of these studies reported no effects [15]. However, Ma and Chu [11] found an effect on developing embryos of the fruit fly (*Drosophila melanogaster* L.), development as well as survival rates were influenced. Also, Fawzia [3] found that the significant frequency of chromosomal aberrations and the micronuclide in the blood of the mice increased after exposing the mice to the electric field.

CONCLUSION

Exposure to extremely low frequency electric fields should be considered as pollutants to the environment, since it has been shown that it affected the properties of the biological cells. Research studies on the biological effects of such fields encourage helping in finding out means of minimizing their hazardous effects.

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EFFECT OF LOW FREQUENCY ELECTRIC FIELD ON GROWTH CHARACTERISTICS AND PROTEIN MOLECULAR STRUCTURE OF WHEAT PLANT

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Abstract. Two exposure systems of an extremely low frequency electric field were used, the first was an experimental model (50 Hz, 6 kV/m strength) and the second was the high voltage transmission lines passing through an open agricultural field (50 Hz, 66 kV/11 m = 6 kV/m). The effects of the two exposure systems were investigated on mitosis, meiosis and pollen grains viability in the wheat plant. Also, the effect of those two fields on some morphological characters and some physiological parameters were estimated. Classification of the water soluble protein (WSP) extracted from the exposed and unexposed grains as well as their molecular weight distributions were also investigated by using SDS polyacrylamide gel electrophoresis (PAGE) technique. The absorption spectra of the WSP were also measured at wavelength range 200–600 nm. The results indicated that the electric field of both systems showed a high frequency of chromosomal abnormalities and the treated wheat flower buds showed a marked increase in the frequency of the nonviable pollen grains. The results also indicated remarkable changes in the morphological characters where the stem length increased but the spike weight and the number of grains in the spike decreased. Further, the data showed an increase in the total chlorophyll content and the total carbohydrates in the grains. On the other hand, the data indicated that the molecular structure of the extracted WSP changed the amount of protein in the bands of exposed grains decreased and their molecular weights changed.

Key words: electric field, wheat plant, cytological, morphological, physiological analysis, protein molecular structure.

INTRODUCTION

The effect of electric field on living cells during decades is mainly attributed to its guide in throwing light on major unsolved biological problems such as morphology, uncoiling immune defense and regulation of the cell division. These electric fields are, practically, produced in all places by numerous sources,

Received July 2006;
in final form August 2006.

THE CYTOLOGICAL ANALYSIS

For mitotic analysis, when the wheat roots reached a length of 1–2 cm, roots were cut and fixed immediately in Carnoy's solution (6 volumes ethanol: 3 volumes chloroform: 1 volume glacial acetic acid) for 24 hours. After fixation the roots were stored in 70% ethanol at 4 °C. For cytological analysis three replicates were performed for each treatment and control. Root tips were macerated in 4% pectinase enzyme (Fluka-0.01 U/mg Chemie AG, CH-9470 Buchs) for 2 hours, then hydrolyzed in 1N HCl at 60 °C for 15 minutes, stained using feulgen squash technique according to Darlington and Lacour [7] and examined microscopically.

For meiotic analysis, flower buds of wheat plants from the two treatments and their control were collected and fixed in acetic acid absolute ethyl alcohol (1:3 v/v) for 24 hours. After fixation the flower buds were stored in 70% ethanol at 4 °C. For cytological analysis, six replicates were performed for each treatment and control, where, the content of the anthers of the flower buds were squeezed out on the slides and stained using the aceto-carmine smearing technique [4].

Also, stain ability of the pollen grains of wheat plants in aceto-carmine stain for the two treatments were performed as an index of determining pollen viability.

All cytological data obtained from the different treatments were statistically analyzed using t-test [21].

THE MORPHOLOGICAL AND PHYSIOLOGICAL MEASUREMENTS

A known fresh weight of leaves from exposed and unexposed grains was taken to define the chlorophyll amount according to Metzaner *et al.* [20].

At the harvest, the plants from each treatment were sampled and the following parameters were measured: stem length (cm), number of spikes per plant, spike weight, number of grains per spike, and weight of 100 grains. Total sugar contents were measured calorimetrically according to Nelson [23] and determination of the total protein content was made [15].

QUALITATIVE ANALYSIS OF WSP

The water-soluble protein (WSP) was extracted in the form of concentrated solution from wheat grains by the method of Irvin [12]. The molecular weights of the components of such WSP of the grains were estimated through the use of SDS polyacrylamide gel electrophoresis (PAGE) according to the technique of Laemmli [14]. The molecular weights of the protein bands were estimated by SDS (PAGE) according to Weber [34]. The gel was stained with Coomassie brilliant blue R-250.

Eight markers of known molecular weights were used as a standard protein, myosin 205 kDa, β-galactosidase 119 kDa, bovine serum albumin 98 kDa, ovalbumin 52.3 kDa, carbonic anhydrase 36.8 kDa, soybean trypsin inhibitor 30.1 kDa, lysozyme 22 kDa, and aprotinin 7.6 kDa.

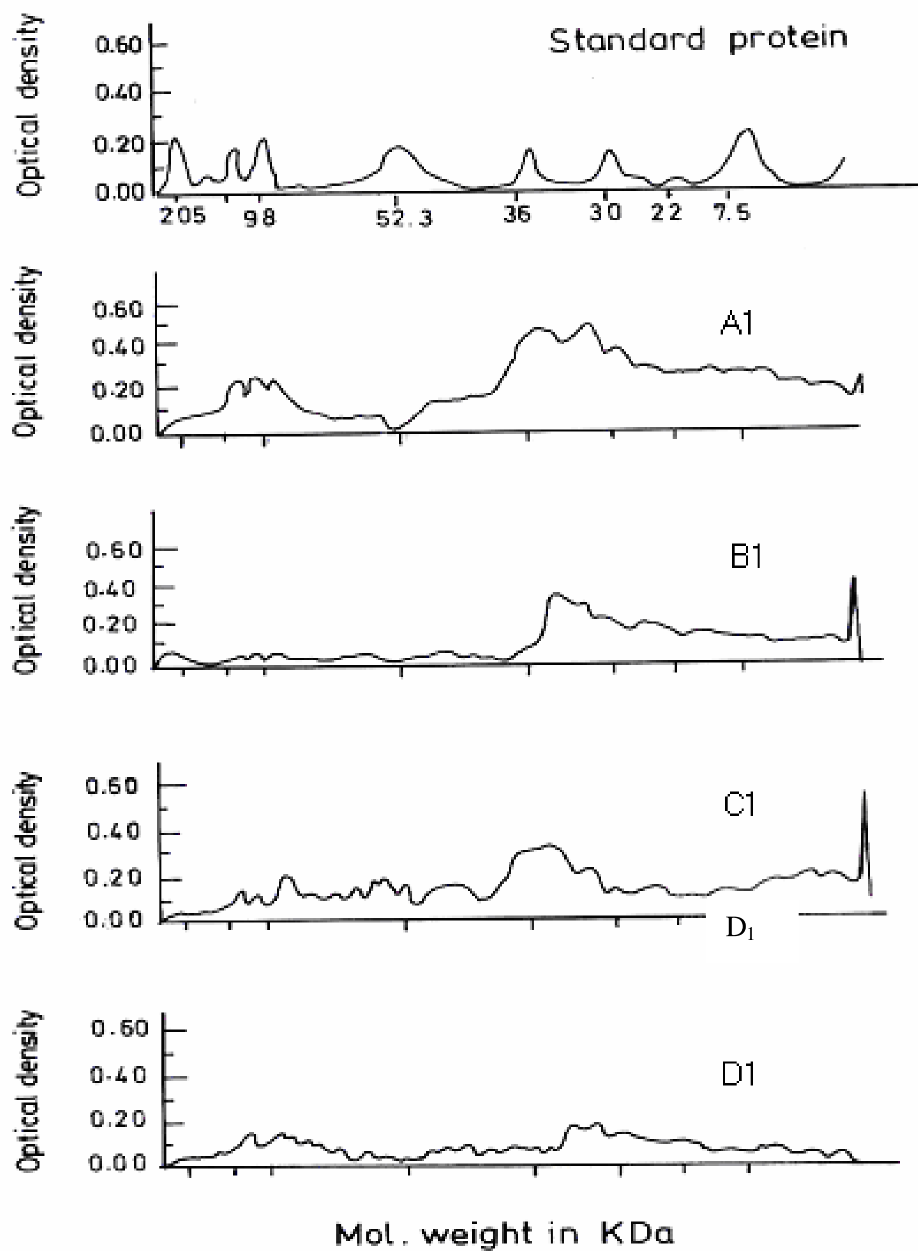

Fig. 6b. The electrophoretic profile of WSP extracted from groups A_1, B_1, C_1 and D_1.

The results of the gel electrophoresis technique of the extracted WSP from the exposed and unexposed grain indicated that the WSP content decreased for the exposed grains (groups B and D) and also its molecular structure changed where the molecular weights of their bands changed; this result is in a good

agreement with Hassan [11], where the changes in the grain protein electrophoretic profiles have been attributed to the occurrence of either gene mutation or indication of cytological aberrations. Also, the increase or decrease in band's intensity in the present study after exposure to electric field can be interpreted on the basis of the gene mutation at regulatory systems that control the concerned structural genes [1, 22]. Also, changes in band's intensity were due to gene duplication resulted from bridges and laggards that are observed at cytological analysis. Therefore, the bridges would lead to gene duplication at one pole and deletion at the other pole, while laggards may be distributed randomly to either poles producing monosomic (gene deletion) or trisomic (gene duplication) cells [1]. In addition, breaks, bridges, laggards and micronuclei may lead to a loss of some genetic material [8]. Disappearance of some bands can be explained also on the basis of protein degradation. These results are supported by the absorption spectra shape where the WSP content decreased by exposing the grains to the electric field (Figs. 7–8).

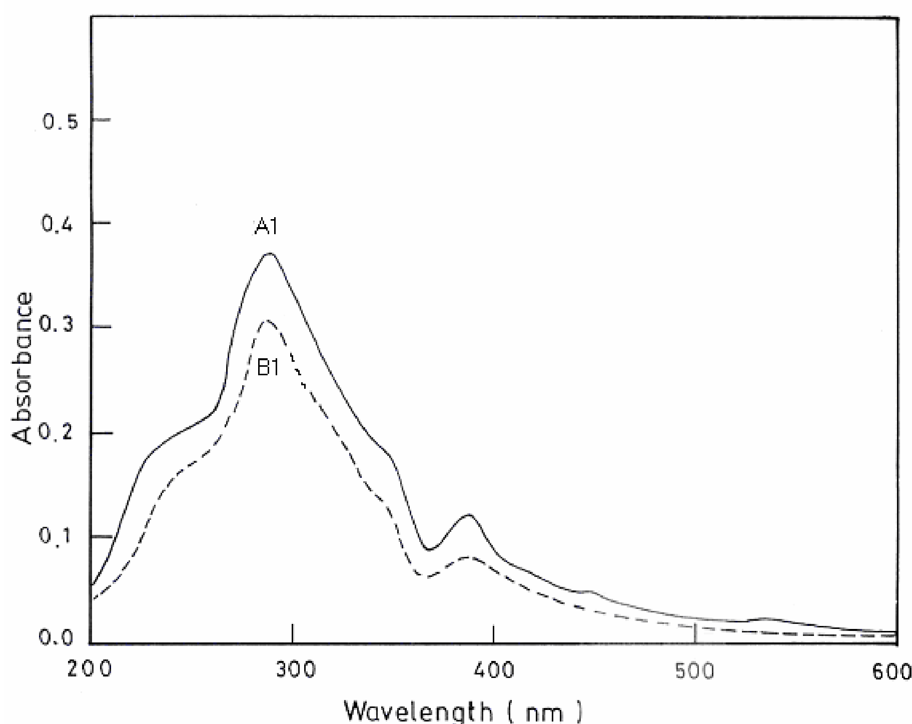

Fig. 7. The absorbance of the WSP of the unexposed grains and grains exposed to electric field group (A_1) and (B_1).

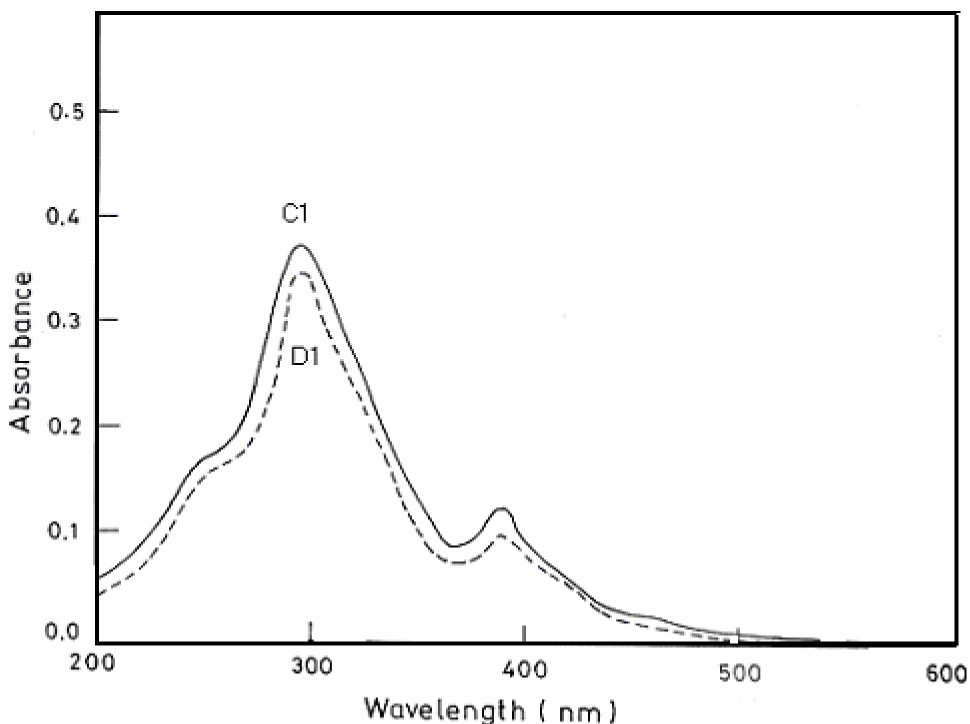

Fig. 8. The absorbance of the WSP of the unexposed grains and grains exposed to electric field group (C) and (D).

CONCLUSION

From the present work, it is concluded that growing plants under high voltage transmission lines change their growth characteristics and protein molecular structure and also decreases plant yield. Therefore, the electric field is considered pollutant to the environment. Hence, it is recommended to insert such transmission lines under the ground to minimize their hazardous effects.

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